

I claim:

1. A method for identifying minicell hosts bound to a binding partner comprising:

(a) expressing a fusion protein in a minicell host comprising an outer membrane, wherein the fusion protein is encoded by a chimeric gene comprising:

a DNA fragment encoding a first peptide which mediates attachment of the fusion protein to the outer membrane, and

a DNA fragment encoding a second peptide;

(b) contacting the minicell host of step (a) with a binding partner; and

(c) identifying the minicell hosts bound to the binding partner.

2. The method of claim 1 further comprising:

(d) isolating the bound or unbound minicell host.

3. The method of claim 1, wherein the DNA fragment that encodes the first peptide mediating attachment of the fusion protein to the outer membrane contains a signal amino acid sequence.

4. The method of claim 3 wherein the signal sequence is selected from the group consisting of ompA signal sequence, ompT signal sequence, ompF signal sequence, traA signal sequence, phoA signal sequence, beta lactamase signal sequence, and the 17K antigen signal sequence from *Rickettsia rickettsii*.

5. The method of claim 1, wherein the DNA fragment encoding the second peptide is from a DNA library.

6. The method of claim 1, wherein the binding partner is selected from the group consisting of carbohydrates, sugars, nucleic acid

molecules, peptides, proteins, metals, inorganic molecules and synthetic drugs.

7. The method of claim 1, wherein the binding partner is selected from the group consisting of receptors, ligands, antibodies, vitamins, cofactors, enzymes, and neuromediators.

8. The method of claim 1 wherein the minicell strain is a gram negative bacteria.

9. The method of claim 7 wherein the gram negative bacteria is selected from the group comprising *E. coli*, *Salmonella typhimurium*, *S. anatum*, *S. enteritidis*, *S. pullorum*, *S. senftenberg*, *S. worthington*, *Vibrio cholera*, *Erwinia amylovora*, and *Haemophilus influenzae*.

10. The method of claim 1 wherein the minicell strain is a gram positive bacteria.

11. The method of claim 9 wherein the gram positive bacteria is *Bacillus subtilis*.

12. The method of claim 1 further comprising:

(d) isolating the DNA encoding the fusion protein; and

(e) subjecting the isolated DNA to analysis methods selected from the group comprising determination of DNA base composition, determination of DNA base sequence, determination of molecular weight, and determination of secondary structures within the sequence.

13. The method of claim 3, wherein the DNA fragment containing a signal amino acid sequence comprises the first 213 nucleotides of the open reading frame of the 17K antigen of *Rickettsia rickettsii*.

14. The method of claim 1, wherein the expression of the fusion protein is controlled by an inducible promoter element.

15. The method of claim 14 wherein the inducible promoter element is selected from a group consisting of lac, tac, and trp.

16. The method of claim 1 further comprising:

(d) cleaving the second peptide from the minicell host.

17. The method of claim 2 further comprising:

(e) cleaving the second peptide from the minicell host.

18. The method of claim 17 further comprising:

(f) isolating the peptide cleaved from the minicell host;

and

(g) subjecting the isolated peptide to methods selected from the group consisting of determination of amino acid composition, determination of amino acid sequence, determination of isoelectric point, and determination of molecular weight.

19. The method of claim 18, wherein the isolated peptide is selected from the group consisting of SEQ ID NO:40, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:52, and SEQ ID NO:53.

20. The method of claim 18, wherein the isolated peptide is selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:49, SEQ ID NO:50, and SEQ ID NO:51.

21. The method of claim 18, wherein the isolated peptide is selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:31, and SEQ ID NO:32.

22. The method of claim 18, wherein the isolated peptide is selected from the group consisting of SEQ ID NO:7, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:35, and SEQ ID NO:36.

23. The method of claim 5, wherein the DNA fragment encoding the second peptide is at least three amino acids in length.

24. A method for increasing diversity of a minicell DNA library comprising mutating the cells in the minicell DNA library and then screening for binding.

25. The method of claim 24 wherein the mutagenesis is *in vivo*.

26. The method of claim 25 wherein the *in vivo* mutagenesis comprises

(a) transformation of an expression vector containing an inducible gene fusion into a minicell strain harboring a mutation in a mut gene; and

(b) adding nucleotides to the minicell strain to induce replication of expression vector.

27. The method of claim 26 wherein the mutation in the mut gene imparts a higher than normal rate of spontaneous mutagenesis on the minicell strain.

28. The method of claim 25 wherein the *in vivo* mutagenesis comprises

(a) transformation of an expression vector containing the inducible gene fusion into a minicell strain harboring a mutation in an aminoacyl-tRNA synthetase gene; and

(b) supplementing the minicell strain with L-amino acids and amino acid analogues to induce protein synthesis.

29. The method of claim 28 wherein the mutation in the aminoacyl-tRNA synthetase gene imparts the ability of the tRNA to recognize, bind, and transfer amino acid analogues.

30. The method of claim 28 wherein the amino acid analogues are selected from the group consisting of hydroxyamino acid, or a derivative thereof, ornithine, azitryptophane, and D-amino acids.

31. A method for screening a minicell DNA library comprising

(a) contacting a minicell library with a target wherein the target is immobilized on solid support; and

(b) separating bound members of minicell library from unbound members, thereby producing a library enriched for DNA encoding a peptide binding the target.

32. The method of claim 31 further comprising

(c) isolating expression vector DNA from the bound or unbound minicells;

(d) mutagenizing the isolated DNA *in vitro*;

(e) transforming mutagenized DNA into minicell strains;

(f) inducing expression of a protein fusion;

(g) contacting the minicell host of step (c) with a target,

and

(h) separating minicells in the library that bound to the target from minicells that did not bind to the target.

33. The method of claim 31 wherein the solid support matrix is selected from the group consisting of agarose, agarose beads, acrylamide beads, cellulose, neutral and ionic carriers, and acrylic polymers.

34. The method of claim 31, wherein the binding partner is selected from the group consisting of carbohydrates, sugars, peptides, proteins, nucleic acid molecules, inorganic molecules and synthetic drugs.

35. The method of claim 31, wherein the binding partner is selected from the group consisting of receptors, ligands, antibodies, vitamins, cofactors, enzymes, metals, and neuromediators.

36. A recombinant expression vector comprising an inducible promoter element genetically fused to an open reading frame fragment of the 17K antigen of *Rickettsia rickettsii*.

37. The vector of claim 36 wherein the open reading frame fragment of the 17K antigen of *Rickettsia rickettsii* comprises a signal sequence.

38. The vector of claim 37 wherein the signal sequence is encoded within the first 213 nucleotides of the open reading frame of the 17K antigen.

39. The vector of claim 38 wherein the 213 nucleotide fragment is genetically fused to DNA encoding a second peptide.

40. The vector of claim 39 wherein expression of the genetic fusion produces a chimeric protein.

41. The vector of claim 36 wherein the inducible promoter element is selected from the group consisting of lac, lacUV5, tac, and trp.

42. A method to make a minicell DNA library comprising

- (a) synthesizing randomized single stranded primers;
- (b) annealing complementary regions of primers to form concatamers; and
- (c) converting single stranded regions of concatamers to double stranded, thereby forming an oligonucleotide library.

43. The library obtained using the method of claim 42, wherein the oligonucleotides of the library comprise at least nine nucleotides.

44. A method for purifying minicells comprising separating minicells from whole cells by density gradient ultra-centrifugation wherein the gradient is made of a linear sucrose gradient.